Microbiome

Human microbiome science: vision for the future, Bethesda, MD, July 24 to 26, 2013

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Abstract

A conference entitled 'Human microbiome science: Vision for the future' was organized in Bethesda, MD from July 24 to 26, 2013. The event brought together experts in the field of human microbiome research and aimed at providing a comprehensive overview of the state of microbiome research, but more importantly to identify and discuss gaps, challenges and opportunities in this nascent field. This report summarizes the presentations but also describes what is needed for human microbiome research to move forward and deliver medical translational applications.

Introduction

Each of us consists of about 40 trillion human cells [1] and about 22,000 human genes [2], but as many as 100 trillion microbial cells [3] (the microbiota) and 2 million microbial genes [4] (the metagenome). Understanding the microbial side of ourselves may therefore be critically important for understanding human biology, including drug responses [5-8], susceptibility to infectious [9] and chronic [10] disease, and perhaps even behavior [11]. Since the inception of the Human Microbiome Project (HMP) in 2007 [4,12], the fundamental understanding of the human microbiome has grown at an ever accelerating pace [13]. Together, the HMP healthy cohort study [14,15], and the many associated studies, which provide more details on methodology, bioinformatics analyses, and additional cohorts, have led to over 350 publications (http://www.ploscollections.org/hmp). This work has set the stage for rapid advances, some with high potential for translational studies, in understanding the mechanisms governing the similarities and differences in the microbes we share, their association with diseases, but more importantly, the functional roles microbiota play in health and disease.

Meeting goals and objectives

To understand the current state of human microbiome research, and to identify key areas for progress going forward, we held a conference in Bethesda, MD from July 24 to 26, 2013, entitled 'Human Microbiome Science: Vision for the Future'. This conference, which was supported in part from a grant by NIH to the University of Maryland School of Medicine, together with corporate sponsors including Roche, Qiagen, Illumina, Life Technologies, MoBio, Metabolon, and the BioMed Central journal *Microbiome*, sought to provide an overview of cutting-edge work in NIH-supported microbiome research, and to identify obstacles as well as opportunities for progress in this challenging field of research. The meeting was organized by a trans-NIH working group, including 28 participants (programme staff) from 14 (of the 27) NIH Institutes, Centers and Offices, together with four scientific advisory members funded by the Human Microbiome Project. The meeting was attended by 269 participants (and an additional 250 with webcast) from academia, national labs, a range of government agencies including NIH, Environmental Protection Agency (EPA), US Department of Agriculture (USDA), Food and

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Drug Administration (FDA), US Agency for International Development (USAID), Office of Science and Technology Policy (OSTP), US Army, National Science Foundation (NSF), and National Aeronautics and Space Administration (NASA), and involved 37 speakers from a broad range of disciplines including microbiology, immunology, medicine, infectious disease, ecology, and computer science. The broad expertise of the organizing committee and the participants underscores the way in which microbes pervade the human body and our environment, and microbiome research may soon pervade the biomedical research enterprise.

Over the course of this 3-day meeting, there were presentations and discussions aimed towards:

- Recognizing that the study of the human microbiome, in disease and in health, is of relevance to the missions of all NIH Institutes and Centers;
- Increasing awareness across all NIH Institutes and Centers of gaps, needs, and challenges faced by the broad microbiome research community to drive future research and investments;
- Facilitating coordination between the NIH Institutes and Centers to promote coherent oversight for policies and approaches that will maximally benefit microbiome-related biomedical research;
- Identifying areas where common resources or partnerships would benefit microbiome-related biomedical research;
- Exploring how NIH and other government funding agencies could collaborate to integrate the microbiome into studies of human health and more broadly into studies of human interactions with their physical and microbial environment;
- Fostering understanding of the current state of microbiome research, and shaping an overall vision for future directions of the field over the next 10 years.

Overview: the human microbiome project

In the first session Dr. Owen White (University of Maryland School of Medicine, Baltimore, MD, USA) (Figure 1A) set the stage for the conference, noting that the meeting was a unique opportunity for microbiome researchers both to reflect on past successes and to define the direction that the field could take going forward. His introduction was followed by a presentation by Dr. Francis Collins (Director of the National Institutes of Health, Bethesda MD, USA) (Figure 1B), who opened with a historical perspective of the Human Microbiome Project (HMP). He presented an overview of how the microbiome is uniquely positioned to enhance the mission of the NIH because of the many associations between the state of the microbiome and a wide range of diseases from

gastrointestinal diseases and conditions, to cancer and even mental illnesses. Dr. Eric Green (Director of National Human Genome Research Institute, NHGRI) (Figure 1C) followed with a report on the state and the many accomplishments of the HMP. The HMP aimed to survey the microbiome in humans through taxonomic and metagenomic analyses. He highlighted the central healthy cohort of volunteers who were intensively sampled, and several demonstration projects that focused on diseases at sites such as the GI track, the skin or the urogenital track. These projects generated over 3.5 Tbp of data and 8 million unique microbial genes were catalogued. These datasets (sequence data, strains, clinical phenotypes, nucleic acid extracts, and even cell lines) are publicly available through repositories and coordinated through a Data Analysis and Coordination Center (DACC) hosted at the Institute for Genome Sciences at the University of Maryland School of Medicine [13]. Overall, the HMP has led to over 350 peer-reviewed scientific publications. The HMP has supported the development of new bioinformatics and technological tools, which altogether facilitate the study of the human microbiome for the scientific community. Human microbiome studies' ethical, legal and social implications (ELSI) were also evaluated, mirroring the Human Genome Project.

Dr. David Relman (Stanford University) (Figure 2A) gave the first of three keynote addresses on 'Diversity, Stability and Resilience of the Human Microbiome', highlighting the larger role the human microbiome plays in both health and disease. He presented recent work from his laboratory on the profound effects of antibiotics in reshaping the human microbiome, and on the value of applying (and perhaps developing) ecological theory for understanding these complex ecosystems and their contributions to human biology [16]. In particular, he raised the issue of resilience, an ecological concept that refers to the amount of disturbance that a system can withstand without changing its self-organizing processes or services [17]. He emphasized the need for better tools to define resilience and evaluate the stability of, and harm to human-associated microbial communities.

Dr. Rosamond Rhodes (Icahn School of Medicine at Mount Sinai, New York) discussed the ethical, legal, and social implications surrounding the study of the human microbiome. In particular, she spoke on issues related to subject identification from knowledge of microbiome sequence data - issues that are in so many ways analogous to those faced by scientists studying the human genome sequence. These issues impact the way microbiome science is and will be performed in the future, especially biobanking. Importantly, they highlight the need to better protect the privacy of subjects involved in microbiome research.

The session was closed by Dr. Robbie Barbero, Office of Science and Technology Policy, (OSTP), who described

Figure 1 Dr. Owen White (A), one of the organizers of the meeting introduced Dr. Francis Collins (B), Director of the National Institutes of Health, who gave an historical perspective on the Human Microbiome Project. Dr. Eric Green **(C)**, Director of the National Human Genome Research Institute discussed the many successes of the Human Microbiome Project. Dr. Jesse Goodman **(D)**, Chief Scientist at the US Food and Drug Administration discussed the regulatory aspects concerning the microbiome, including the challenges associated with fecal transplants.

the role of OSTP in supporting cross-agency collaboration to meet 'Grand Challenges' that address important societal needs with a combination of research, technology, and policy inputs. He described several successful Grand Challenges to date, and asked the microbiome community to consider whether a microbiome-focused Grand Challenge might be appropriate at this point in time, especially given the pervasive impact of microbes in the environment and in our bodies.

Basic biology of the microbiome

This session consisted of three exciting talks describing what we know about how the microbiome develops and changes over time, elaborating on the theme developed earlier by Dr. David Relman. Dr. Ruth Ley (Cornell University) described the changes in the gut microbiota throughout pregnancy, as well as those experienced by newborns in the first few years of life [18]. She presented

exciting new work that aims at linking host genetic variations and the composition of the human microbiome. For example, in a cohort of more than 1,000 twin adults, heritable genera of Bacteria and Archaea were identified. Understanding the contribution of human genetic varibility in shaping the composition of the human microbiome will govern the way we will be able to manipulate these microbial communities to maintain or restore health and cure diseases.

Dr. Jacques Ravel (Institute for Genome Sciences, University of Maryland School of Medicine) discussed the ecological principles that govern the dynamics of the human microbiome (resilience, resistance and persistence) and how one can gain better understanding of these dynamic systems using descriptive microbial community compositional surveys [19], gene composition and whole community gene expression or even metabolite analysis. Each of these analyses often reveals different intrinsic

Figure 2 Keynote speakers. Dr. Jonathan Braun, University of California Los Angeles, **(A)** highlighted the challenges in translating microbiome sciences. Dr. David Relman, Stanford University, **(B)** discussed the larger role the human microbiome plays in both health and disease. Dr. Maria Dominguez-Bello, New York University, **(C)** discussed aspects of the modern versus ancestral microbiome.

dynamic patterns when applied to the same community. His presentation stressed the pitfalls that could result from *a priori* application of principles that might govern microbial community at a given body site to another site. Discussing the vaginal microbiota he introduced a new concept that the intensity of dynamic changes (that is, frequency and duration of change in microbiota composition) could represent an increased risk for acquisition and transmission of sexually transmitted infections. Dr. Frederic Bushman (University of Pennsylvania) then spoke about his work on understanding the dynamics of the human gut virome (the set of viruses that targets bacteria and humans). Although immense progress has been made in understanding the bacteria that inhabit the human body, especially through 16S rRNA gene sequencing and reference genome approaches, studies of the human virome have lagged behind. Analysis of the composition of the gut virome shows that, at least over 2.5 years, the dynamics of the virome is subject-specific and may be important for predicting health, and that the vast majority of viral diversity in the human gut is still uncharacterized and rapidly evolving [20,21]. These studies suggest that a targeted effort to understand viral diversity may be needed given their likely importance for the gut ecosystem as a whole.

State of the art microbiome tools, technologies, approaches

Microbiome studies rely on the development of novel and robust technologies, approaches and analytical tools. Dr. Janet Jansson (Lawrence Berkeley National Laboratory) described cutting-edge research that combines multi-omics approaches for in-depth characterization of gut community function in health and disease. Taking examples from her own work and those of environmental scientists, she demonstrated the limitations of 16S rRNA gene-based community survey which often shows high variation between subjects, while functionally the communities are more similar. She advocated for applying metaproteomics combined with metabolomics to gain more accurate insights into the function of the community and often the host as well [22,23]. The addition of these multiple layers of omics data provides substantially greater insight into possible mechanisms, and when joined with time-series data can be critical for understanding which differences are causes and which are effects of processes at other levels. However, she stressed that computational tools for functional assignment and for omics' data integration are still in their infancy and desperately needed. Dr. Dan Littman (New York University) spoke about approaches for studying the interaction of the host immune system and the microbiome, noting the importance of specific commensals in inducing inflammatory T cell responses [24]. He specifically highlighted an association of *Prevotella copri* colonization with the autoimmune response in rheumatoid arthritis [25,26]. Dr. Curtis Huttenhower (Harvard School of Public Health) described novel bioinformatics tools for reconstructing the biomolecular networks driving emergent phenotypes in the microbiome and their influences on human health [27]. These computational methods provide the initial steps to integrate multi-omic data, generate mechanistic hypotheses, and identify actionable molecular targets for therapy. He also discussed the need for improved study designs to effectively scale microbiome investigations to epidemiological populations, which along with principled methods for meta-analysis will aid in ensuring reproducible translational results.

Dr. Rob Knight (University of Colorado at Boulder) described the challenges in moving from associative studies that link microbes to disease towards studies of causality using either germ-free mice models or epidemiological criteria for causation. He also discussed the challenges of integrating human microbiome datasets that use different methodologies and different subject populations. He discussed the critical aspect of the effect size of each metadata element. For example, because age and body site can have large effects even in studies that use very different methods, it is essential to control for technical variability when examining serial samples from the same patient or sub-site analysis (for example, stool *versus* lumen). He also showed the utility of the HMP dataset [14,15] as a data frame for integrating dynamics of time series datasets such as during infant development and for understanding remission of *Clostridium difficile*-associated disease after fecal microbiota transplantation (FMT). Dr. Owen White (University of Maryland School of Medicine) closed the session by discussing integration of large datasets such as those from Human Microbiome Project into accessible resources such as the HMP DACC, which he leads [13]. He emphasized the need for adoption of standards such as those developed by the Genomic Standards Consortium to enable systematic analysis of these integrated datasets [28]. At present, privacy concerns make it difficult to obtain and harmonize data from different projects, especially because a considerable amount of data resides in dbGAP (the access-controlled Database of Genotypes and Phenotypes). He stressed that open resources will go a long way towards making the controlled-access data more reusable. Finally, he described OSDF (the Open Science Data Framework); an open-source project that provides methods for accessing large volumes of data on distributed file systems, including cloud computing resources, which are increasingly gaining importance as the volume of data expands.

The first day concluded with an open discussion facilitated by Ed Young, a science writer and blogger, between the day's speakers and the conference participants. A wide range of topics was covered including: the potential of

microbiome data to impact health; the need for improved reusability of the resources generated through investment by NIH and other agencies; and the importance of expanding microbiome studies to understand the functional role of the microbiome in disease. To date, most microbiome studies have mostly been associative (associate one or more organisms with a disease state), and the need for studies that are designed to address causality was also discussed. Many audience members were interested in issues of subject identifiability via the microbiome and how a microbiome can be changed in a specific desired direction; both of these topics are areas of intense scientific interest, however, at present a clearer picture is still emerging.

The modern *versus* the ancestral microbiome

In the second keynote, Dr. Maria Gloria Dominguez-Bello (New York University) (Figure 2B) spoke about the modern versus ancestral microbiome [29,30]. She described how modern practices including hygiene, antibiotics, and limited exposure to livestock have likely affected the composition of the human microbiome. She showed that people living more ancestral lifestyles, without antibiotics and vaccines, such as a previously uncontacted group of the Yanomamö Amerindian tribe in Venezuela, have a profoundly different microbiome as compared to western peoples. Their microbiomes exhibit high diversity not just in the gut, but at all body sites surveyed. She concluded her presentation with a description of a fascinating project that aims to collect not just biological samples, but environmental samples including household surfaces, water, soil, air, domestic pets and livestock along gradients of Westernization in both South America and Africa. Such studies may be critical for understanding the role of microbes, or their loss, in several so-called 'Western diseases' which are a high cost burden on the US health system.

Host immune system/microbiome interactions

Dr. Sarkis Mazmanian (Caltech) discussed how specific bacterial genes control how some gut bacteria colonize the intestinal tract [31]. Focusing on *Bacteroides* species in mice, he presented data demonstrating that a unique class of microbial polysaccharide utilization loci is responsible for species-specific saturable colonization of the crypt channels in the gut. These commensal colonization factors (ccf) loci in *Bacteroides fragilis* and *Bacteroides vulgatus* enable species-specific physical interactions with the host that mediate stable and resilient gut colonization; ccf mutants are defective in horizontal transmission. These studies stress the importance of species-specific genes that, if absent, could affect the establishment of a healthy microbiome. Dr. Eugene Chang (University of Chicago) discussed the effects of the gut microbiome on host epithelial functions and responses [32,33], focusing on the pouchitis model that has been extremely informative in his HMP demonstration

project. Dr. Susan Erdman (Massachusetts Institute of Technology) presented her work on animal models showing that specific microbial exposures affect host hormones, including oxytocin [34], interrelated with host immune cell functioning. She showed that prior exposures to gut microbes alter the immune system and potency of T regulatory (Treg) lymphocytes, lowering risk for systemic diseases including cancer later in life. These studies highlighted microbe-endocrine-immune linkages and possible mechanisms for transmitting the effects of maternal microbial exposures to offspring, including behavioral benefits such as improved social interactions.

Microbiome and disease associations

Dr. Heidi H Kong (NIH, NCI, Dermatology Branch) discussed how skin microbiota can influence host skin immunity and vice versa. For example, Yasmine Belkaid and co-workers [35] showed that applying the skin commensal *S. epidermidis* on germ-free mice can restore the ability to control skin infections by the parasite *Leishmania major*. These findings have potential implications for the development of rational tissue-specific adjuvant and vaccine approaches. In addition to highlighting the importance of studying fungal communities as well as bacterial communities, she discussed work demonstrating the alterations of the skin microbiome in patients with atopic dermatitis (AD) and primary immunodeficiencies [36]. The notable differences in the skin microbiomes of these patient populations may reflect how the host can modulate its skin microbiome and potentially elicit episodes of skin disease. Since AD is often linked with asthma and hay fever, understanding the triggers of AD may allow scientists to develop strategies to prevent and treat these other disorders. Dr. Gary Huffnagle (University of Michigan) described the major challenges associated with sampling the lungs, a body site previously believed to be sterile [37,38]. It is now known that a low-abundance microbiota exists in the lung and that when diseased, the microbial load increases, comprising numerous taxa not found in the mouth or throat. This indicates that there are selective pressures in the lungs for bacterial persistence, colonization and growth that uniquely shape the bacterial community of this body site. Dr. Vincent Young (University of Michigan) reviewed Koch's Postulates and their implications for moving beyond a 'classical' infectious disease model and towards an understanding of the role of both commensal and pathogenic microbes in the development of inflammatory bowel disease. In particular, understanding the interactions of normal gut bacteria with the host mucosa is critical to understanding how dysregulation of these normal interactions can trigger the abnormal host response that characterizes inflammatory bowel disease [32].

Functional interactions between host and microbiome

Microbes are important modulators of host phenotypes. It is critical to better understand the mechanisms governing this modulation. Dr. Andrew Neish (Emory University) discussed how the microbiome controls epithelial cell proliferation, focusing on the role of microbes in stimulating reactive oxygen species (ROS) production in the gut epithelium [39,40]. In Nox1 and Frp1 null mice, and dNox knockout *Drosophila,* the dynamics of crypt cell proliferation are substantially altered, suggesting that signaling to host cells via reactive oxygen species stimulated by commensal bacteria is important. Dr. Peter Turnbaugh (Harvard University) spoke about the impact of gut microbes on drug metabolism; for example, how certain strains of the gut bacterium *Eggertella lenta* carry a cytochrome operon that causes inactivation of the cardiac drug digoxin [7]. These and other studies highlight the important role of the microbiome in altering the outcome of therapeutics. A better understanding of these interactions may someday allow us to devise strategies to improve drug efficacy and reduce side effects. Dr. Wendy Garrett (Harvard School of Public Health) discussed how microbial metabolites, in particular short-chain fatty acids which are the major end products of bacterial fermentation of dietary polysaccharides, regulate the size and function of the colonic Treg pool, and can protect mice against colitis [41,42]. Thus, short-chain fatty acids may act as a transducer of the gut microbiome into a reliable signal that can regulate immune homeostasis and function in the colon.

Diet and the microbiome

Dr. Ian Jeffery (University College Cork, Ireland) presented the ElderMet project that studies diet-gut microbiota interactions as it relates to the health of the elderly [43]. In this project, comparisons of elderly subjects living in the community, residential-care or hospital settings were performed using a combination of dietary assessment, gut microbial characterization using 16S rRNA gene surveys, and NMR-based metabolomic analyses. Differences in diet and living situation were highly correlated with differences in the gut microbiota composition and function. Within these populations the microbiota was associated with health outcomes in the individuals such as frailty and inflammatory markers. Over the long term, diet was associated with changes in gut microbiota and therefore dietary modulation of the microbiota may have an impact on health of the elderly. This work could lead to carefully designed dietary interventions to promote healthier aging. Dr. Kathryn Dewey (University of California, Davis) reviewed what is known about the influence of diet in early life on the microbiome. She discussed the key role of breastfeeding, noting that the microbial composition of breast milk may be influenced by the mother's weight and mode of delivery, and that prebiotics in human milk promote the growth of beneficial gut bacteria. Although many studies have compared breast-fed to formula-fed children, it is still unclear which specific aspects of breastfeeding have an effect on the gut microbiome. Introduction of solid foods, the types of solid foods consumed, and certain nutrients such as iron and fatty acids influence the diversity and composition of gut bacteria. However, there is little information on how dietary composition or nutrient intake affects the microbiome of children and the health consequences of differences in the gut microbiome. A new project entitled the Breast Milk, Gut Microbiome, and Immunity (BMMI) Project aims to discover new ways to promote healthy growth in infants and children, and will address some of these important questions. Dr. Johanna Lampe (Fred Hutchinson Research Center and University of Washington) discussed how a range of dietary components are metabolized by bacteria, potentially impacting human health [44]. For example, bacterial metabolites can act as nutrients for host cells (for example, short-chain fatty acids), act as signaling molecules, or be genotoxic (as in the case of nitrites and hydrogen sulfide) or beneficial to host cells (for example, isothiocyanates and flavonoids). Of particular interest in studies of cardiovascular disease is the bacterial conversion of choline to trimethylamine, which is subsequently converted to trimethylamine N-oxide (TMAO) in the liver.

Translational research and the microbiome

The third day was dedicated to strategies for moving beyond basic association between microbiome and disease by exploiting the microbiome to improve human health. The third keynote was given by Dr. Jonathan Braun (UCLA) (Figure 2C) who noted that the key to translating microbiome science was to identify plausible mechanisms to explain how bacteria might affect the host, as well as therapeutic targets for modulating bacterial activity. He highlighted a number of diseases that were either caused by pathogenic microbes or by a 'pathogenic' microbial ecosystem, including *Clostridium difficile*-associated colitis, inflammatory bowel disease, Type 1 diabetes, lymphoma, atherosclerosis, and elements of behavior and cognition. The main challenges are to untangle the relationships among the complex networks of microbial species, their functions and products and how they mediate effects on the host (and vice versa) [45]. Understanding the properties that drive these networks is key to designing reliable interventions. This presentation was followed by Dr. Jesse Goodman (FDA, Chief Scientist) (Figure 1D) who covered regulatory aspects concerning the microbiome, in particular the FDA's decision to require an IND (Investigational New Drug) application approval for all FMT other than those for treating *C. difficile* infections. In his presentation he also reviewed the regulatory issues surrounding probiotics not covered by the

GRAS (Generally Recognized as Safe) guidelines, for example, microbial strains isolated from traditionally consumed fermented foodstuffs. He stressed that the FDA's mission was to get effective therapies and diagnostics into the hands of consumers as rapidly as possible, however, within the microbiome space methods for demonstrating safety and efficacy are still in their infancy and very much evolving.

Body/microbiome axis

Dr. Ted Dinan (University College Cork, Ireland) discussed his work on the microbiome-gut-brain axis where he uses germ-free mice to demonstrate that the lack of gut microbes affects sociability, decreases memory, and increases stress responses. He discussed the role that bacteria play in producing neurotransmitters, such as norepinephrine, serotonin, or dopamine, as well as how certain probiotic bacteria can actually modulate the effects of neurotransmitters. In particular, he presented data showing how specific strains of *Lactobacillus rhamnosus* modulate stress and this effect appears to be mediated through the vagus nerve in mice [46]. He cautioned that identifying psychobiotics (a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness) should involve rationally designed screening strategies of very large numbers of putative microbial strains, and that, just as is the case with chemical drugs, most strains are expected to have no effect on most disorders. Dr. Martin Blaser (New York University) spoke about both epidemiological evidence linking antibiotic use and the risk for obesity [47]. He presented experimental evidence in mice that sub-therapeutic antibiotic treatment as well as pulsed full-dose antibiotic treatment modifies body composition, growth, and immune status, leading to increased adiposity. These results, together with the use of antibiotics as growth promoters in livestock, suggest that the obesity epidemic in humans may be in part attributable to modifications of the gut microbiota by antibiotics, especially in early in life. Dr. Stanley Hazen (Cleveland Clinic) discussed links between microbes and cardiovascular disease, and in particular the role of bacteria in converting choline to TMAO, which in turn promotes atherosclerosis [48]. This model, supported by careful work in mice and in human subjects, suggests a direct and specific mechanistic link between gut microbes and cardiovascular disease. Dr. Christian Jobin (University of Florida School of Medicine) presented his work on gut microbiome and colorectal cancer [49], stressing the importance of mechanistic studies identifying specific bacterial genes involved in the causal pathway to cancer. Using IL10-/- germ-free mice which develop colitis-associated colorectal cancer after exposure to microbes, he identified the polyketide colibactin, produced by *E. coli*, which appears to play a substantial inflammation-dependent role in colorectal cancer development in mice and humans. These studies have started to untangle the role of inflammation, gut microbiota, and specific bacterial genes in the development of cancer. Dr. Julian Davies (University of British Columbia, Canada) ended the session with a discussion of the wealth of chemical products that microbes produce, and of the importance of mining this wealth using rational genomic approaches for identifying microbial secondary metabolites with therapeutic activities. He noted that the microbiome itself represents a great source of novel and potentially bioactive natural products [50].

Probiotics, microbiome vaccines, and fecal transplants

The final session turned to methods for directly manipulating the microbiome. Dr. Alexander Khoruts (University of Minnesota) covered the remarkable efficacy (approximately 90% remission rates) of fecal microbiota transplantation (FMT) in curing recurrent *Clostridium difficile* infection (CDI). The potential mechanisms of action are starting to be elucidated and involve ecological, immunological, and metabolic (bile acids) components - all are proposed to lead to *C. difficile* colonization resistance. He noted that many challenges remain before widespread implementation of FMT can become a reality in routine clinical practice. Dr. Elaine Petrof (Queen's University, Canada) provided a complementary approach for FMT using synthetic stool made of defined communities of bacteria cultured from human feces but grown in the laboratory, rather than samples from donors. She described the successful treatment of two CDI patients using a defined synthetic stool comprising 33 bacterial isolates [51]. This approach holds substantial promise for improving patient and physician acceptability of FMT. Dr. David Mills (University of California, Davis) discussed the role of milk-oriented prebiotics and probiotics in identifying compounds and bacteria that could form the next generation of rationally-designed prebiotics and probiotics to improve and support infant health. He described the rich diversity in oligosaccharides found in human breast milk and noted that the types of glycans found in breast milk help to shape the composition of the infant gut microbiota, specifically different species of *Bifidobacterium* [52]. This information could be used to design synbiotic formulations comprised of specific human milk oligosaccharides and bacteria to drive a healthy infant gut. Ultimately, understanding the co-evolution of milk glycans, the immune system, and gut bacteria in infancy may be critical in improving human health in infants and may be among the first translational models for modulation of the gut microbiota. Finally, Eric Brown (representing the Brett Finlay Lab, University of British Columbia, Vancouver, Canada) spoke about gut microbiota and vaccine efficacy. In developing countries vaccine

efficacy is lower and the gut microbiota is different from that in developed countries. He suggested a link between the impact of diet and malnutrition on the composition of the gut microbiota and vaccine efficacy. He highlighted a need for studies to explore ways to manipulate the gut microbiota to improve vaccine response, including probiotics and/or prebiotics. Understanding the basis for vaccine failure in developing countries is a key issue in global public health. Further, developing tests for the maturation of the infant microbiota and immune system, which could be used to improve public health strategies and vaccine efficacy could have a large beneficial effect.

The meeting closed with another floor discussion moderated by Ed Yong, which gave participants a final chance to share their thoughts about the workshop and about the future of human microbiome research. He led discussions on the impact of antibiotics on the gut microbiota and long-term health, as well as on ways to better understand mechanisms of action driving the benefit associated with manipulation of the microbiota.

Gaps, needs, and challenges: a framework for the future of human microbiome studies

A key objective of this meeting was to identify gaps, needs and challenges specific to each individual research project presented, and the field as a whole. In addition, the meeting organizers aimed to expand microbiome research by including specialists in other disciplines who could benefit from a microbiome focus. Not surprisingly, speaker responses to the questions of gaps, needs, and challenges varied, nevertheless, some themes emerged:

Causation and the need for prospective longitudinal studies

A challenge in microbiome research is to move beyond identification of microbiota community structures that correlate with disease states to establishing a causal link between structural changes and the functions of microbiota in disease. Prospective longitudinal studies in humans were recommended to help better understand the drivers of microbiome dynamics with respect disease risk and to develop predictive models of susceptibility that could suggest better health practices (Relman, Ravel). Prospective long-term follow up of intervention trials are also needed to identify the consequences of differences in microbiome structure and function in early life (Blaser, Dewey, Knight). In mice, multi-generational studies that monitor transfer of microbiota to offspring over generations with changes in diet, antibiotics, or environment are important for testing cause/effects relationships between allergic, autoimmune, and behavioral disorders and the microbiome (Mazmanian). Improved clinical phenotyping

and clean study design are essential to successful microbiome studies (Chang). Understanding links between host genetics and the microbiome may also require a longitudinal component, as some phenotypes develop only at specific ages (Ley).

Improved understanding of microbial ecosystems of the human body

We need more robust knowledge of ecological networks within microbial ecosystems and their stability over time within individuals (Braun). Characterization of microbial succession and colonization, and of the natural history of microbes and the disorders they cause, was noted as a potential means to correct dysbiosis or influence metabolism of drugs (Mazmanian, Ley, Turnbaugh, Chang). Relman raised several key questions, including: which aspects of diversity matter most, and do these aspects mainly occur at the level of organisms, genes, or pathways, or between communities? He argued that understanding the fitness landscape of an individual would improve our knowledge of resilience and contribute to strategies for maintenance and restoration of important ecosystem services. Mazmanian and Knight also noted that understanding specific and successful colonization processes would be critical to improve disease outcomes.

Host-microbiome signals and interactions

Presenters noted our poor understanding of the signaling and communication processes between microbiomes and the host. Improved methods and models systems are needed. While recognizing the limitations of animal models, they nevertheless have provided a wealth of information that have led to our current understanding of the role of the microbiome in health and disease and continue to generate novel hypotheses that can be tested in well-designed human studies. Continued support for the development of better animal models is critical to the future of this field. Studies that investigate the mechanisms by which the microbiota influences and is influenced by the immune system (Littman, Garrett, Chang), or how hormones, such as estrogen impact the vaginal ecosystems (Ravel), are desperately needed. Exhaustive identification of small molecule, bioactive metabolites, secreted and cell surface peptides that can influence microbe-to-host interactions, would be highly desirable (Huttenhower, Mills, Jobin).

Analysis approaches and tools

Many concerns regarding the lack of tools for the analysis of 'omic datasets being generated by the microbiome community were expressed during the meeting. Researchers need improved methods to perform quantitative measurements of transcripts, proteins, and metabolites (Braun, Ravel). There is also a lack of consensus as to how much

diverse 'omics' data is needed for robust scientific interpretation (Knight). The need for better methods to integrate large and diverse 'omics' datasets was frequently mentioned (Young, Jeffrey, Lampe, Jansson, Huttenhower, Knight) and the sheer volume of information should be considered to be 'big data' problem (Jansson). The lack of procedures to integrate multiple omics data types in longitudinal studies was also identified (Knight). The overall need for increased interdisciplinary collaboration to generate and interpret data was noted (Mills, Lampe, Jansson), mainly because of the different expertise needed to analyze such datasets exceeds what any individual lab can do. Presenters pointed out that tools for the analysis, visualization, and manipulation of these large datasets are also needed. Users would like, for example, to 'make meta'omics analysis as easy as microarray analysis' (Huttenhower). Specific systems for analysis of metabolomic, genetic, glycomic datasets (Mills), or sequences from low-biomass samples (Kong), and mass spectrometry data (Davies) are also lacking. Many presentations at the meeting thus revolved around the generation of diverse 'omics' datasets, and the challenges associated with integrating that information.

Standards

Several presenters expressed that more standards are needed in microbiome research. Standardized protocols improve reproducibility of microbiome experiments and ensure translation of results from independent experiments (Braun, Huttenhower, White, Knight). White suggested that software could be developed to improve the uniformity of data submissions to the NCBI Short Read Archive and dbGaP. Further, uniform clinical and laboratory procedures such as sampling methods at different body sites, PCR protocols, and DNA/RNA extraction methods would also improve our ability to compare data from different research projects (Kong). It was also noted that non-standardized diets for model organisms could account for phenotypic differences between experiments (Mazmanian). Presenters also cited the challenges associated with non-standard metadata associated with microbiome work. Most sample information is not in a standardized format (Knight), and standardized clinical description of phenotypes is lacking (Kong). A solution to these metadata issues would be to utilize software systems similar to the PhenX toolkit for describing common clinical data elements [53], in combination with standardized metadata deposition practices being enforced by scientific journals (White). There was a clear need expressed for collection of data and tools in a single accessible site, much as the HMP DACC provided for the Human Microbiome Project.

Conclusions

The meeting was clearly a success, in that it highlighted the amazing progress in microbiome research funded across a range of NIH Institute and Centers, focusing on a wide array of diseases. Additionally, while the speakers responses to the needs, gaps, and challenges varied, themes focusing on a few key areas emerged: studies of causality (mechanistic studies in model organisms and prospective longitudinal studies), need to integrate more complex omics and phenotype data, and better standardization of methods and data. Overall, the diversity and excellence of talks underscored how much has been done in this field, in just the past 6 years, and the potential for microbiome science to produce a revolution in human health.

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